

## **Growth Characteristics and Stress Response Parameters of Yeasts Treated with Fungicidal Secondary Metabolites**

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Fungal infections are of increasing relevance in immunocompromised patients and *Candida albicans* plays a dominant role among the human fungal pathogens. This budding yeast has diverged from *Saccharomyces cerevisiae* lineage several million years ago, so that significant homologies but also differences in gene and protein functions can be expected. We follow a comparative strategy between both yeasts to elucidate molecular pathways and mechanisms underlying the mode of action of fungicides, as *S. cerevisiae* is much better analysed and understood than *C. albicans*.

We chose as test compounds secondary metabolites from bacteria with proven fungicidal properties, but an unknown molecular target. Methylglyoxal, a metabolite derived from glycolysis of bacteria, fungi and even mammalian cells, pyrrolnitrin and ambruticin, both secondary metabolites from myxobacteria, were reported to lead not only to growth inhibition but also to glycerol production in sensitive strains.

Glycerol production is observed as byproduct of metabolic fermentation pathways and as a consequence of hyperosmotic growth conditions. During fermentation glycerol is transported out of the cells and thus it is found in the medium, whereas osmotic stress leads to a closure of the glycerol channels and thus, a significant amount of glycerol is found intracellularly. Thus, we determined the dynamics of intracellular and extracellular glycerol production. These data were supplemented by the quantitative determinations of extracellular ethanol, intracellular NADH and cAMP (cyclic adenosine monophosphate) and intracellular activities of alcohol dehydrogenase and aldehyde dehydrogenase.

Ambruticin showed no growth-reducing and glycerol inducing activity on *S. cerevisiae*, even when mutant strains were used, and even 3 out of 5 *C. albicans* wild-type strains proved to be resistant. The other two compounds reacted on both yeasts. We confirmed the intracellular accumulation of glycerol after treatment with ambruticin only in sensitive strains, whereas treatment with methylglyoxal led to a rapid increase only of extracellular glycerol. The kinetics and amount of glycerol induction due to compound treatment usually differed from induction due to osmotic stress. Related to glycerol induction is a reduction of ethanol production, in particular after methylglyoxal treatment. Concentrations of cofactors, such as NADH or cAMP, were hardly affected by compound treatment.

Even, if observations were similar and seemed to follow the same general principles for both yeasts, differences in the kinetics of the responses and in the resulting concentrations of metabolites were observed.